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Leaf scarring by the weevils *Neochetina eichhorniae* and *N. bruchi* enhances infection by the fungus *Cercospora piaropi* on waterhyacinth, *Eichhornia crassipes*

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Abstract. Additive or synergistic effects among introduced and native insect and plant pathogen agents are necessary to achieve biological control of waterhyacinth (*Eichhornia crassipes*), a globally damaging aquatic weed. In field plots, plants were infested with waterhyacinth weevils (*Neoechetina bruchi* and *N. eichhorniae*) and leaves were scarred by weevil feeding. Subsequent infection by the fungal pathogen *Cercospora piaropi* caused necrotic lesions to form on leaves. Necrosis development was 7.5- and 10.5-fold greater in plots augmented with both weevils and *C. piaropi* and weevils alone, respectively, than in plots receiving only *C. piaropi*. Twenty-four days after weevil infestation, the percentage of laminar area covered by lesions on third-youngest and oldest live leaves was elevated 2.3–2.5-fold in plots augmented with weevils. Scar density and necrosis coverage on young leaf laminae were positively correlated, even though antipathogenic soluble peroxidases were elevated 3-fold in plots augmented with weevils alone or weevils and *C. piaropi*. Combined weevil and fungal augmentation decreased shoot densities and leaves per plant. In a no-choice bioassay, weevil feeding on oldest but not young leaves was reduced 44% two weeks after *C. piaropi* inoculation. Protein content and peroxidase activities were elevated 2–6-fold in oldest leaves three weeks after inoculation. Augmentation with both waterhyacinth weevils and *C. piaropi* led to the development of an additive biological control impact, mediated by one or more direct interactions between these agents, and not plant quality effects.

Key words: additive effects, aquatic weed (Pontederiaceae), augmentation, bioherbicide, biological control of weeds, Curculionidae, plant defense, Texas, USA

Introduction

Success in weed biological control often requires the release and establishment of multiple agents exerting cumulative impacts (Syrett et al., 2000; Denoth et al., 2002). Associations among weed biological control

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agents may arise if infestation by one agent directly alters the ability of others to infest the target (Caesar, 2003) or if attack alters target plant quality, indirectly influencing the feeding, survival and/or reproduction of other agent(s) (Milbrath and Nechols, 2004). Positive interactions between insect herbivores and plant pathogenic fungi are potentially useful in biological weed control, but are seldom studied mechanistically (Zidack, 1999; Caesar, 2000, 2003; Muller-Scharer et al., 2000; Charudattan, 2001). In crops and other plants, insect feeding wounds provide entry points for fungal pathogens, and insects can deliver fungal inoculum on cuticular surfaces or in digestive excreta (Hatcher, 1995; Paine et al., 1997; Caesar, 2003). Infection by fungi produces foliar necrosis symptoms and often induces changes in plant proteins, sugars, and other nutrients (Hatcher, 1997) and defensive enzymes such as peroxidases (Hammerschmidt and Kuc, 1995). Disease symptoms and altered biochemical profiles can influence insect feeding behavior, growth, survival, and reproduction (de Nooij et al., 1992; Hatcher, 1997).

Aquatic weeds are attacked by native and exotic fungal plant pathogens that cause necrosis on leaves and stems (Barreto et al., 2000), and introduced insects that wound leaves by surface chewing or tunneling (Forno and Julien, 2000). Biological control of waterhyacinth (*Eichhornia crassipes* (Mart.) Solms.) has involved the worldwide release of four arthropods (the weevils *Neochetina bruchi* Hustache and *Neochetina eichhorniae* Warner, Coleoptera: Curculionidae; the moth *Niphograpta albiguttalis* (Warren) (Lepidoptera: Pyralidae); and the mite *Orthogalumna terebrantis* Wallwork (Acarina: Galumnidae), and several other agents have been released in some areas or are under development (Center et al., 2002). At least four fungal pathogens been studied as promising candidates for mass-production and release or augmentation, or are in use in specific areas (Charudattan et al., 1985; Charudattan, 2001). Feeding by *Neochetina* spp. waterhyacinth weevils reduces plant biomass and reproduction and increases shoot mortality (Center et al., 1999b). A native fungal pathogen, *Cercospora piaropi* Tharp, occurs throughout the weed's range in the southeastern USA (Freeman et al., 1981; Charudattan et al., 1985). Field studies (Addor, 1977; Charudattan, 1984) have revealed a positive association between *Neochetina* spp. weevil feeding and *C. piaropi* infection, which may lead to additive or synergistic increases in plant mortality (Charudattan, 1984) or no effects on biocontrol (Cofrancesco et al., 1985; Center, 1987). As in other weeds (Caesar, 2000), the causes and consequences of the weevil-fungus association are poorly understood.

In the Rio Grande Valley of South Texas, adult *Neochetina* weevil feeding on the laminae of young leaves is positively correlated to *C. piaropi-*

induced necrosis on old leaves, and both types of damage are present at most field sites (Moran, 2004). In the present study, experimental infestation and infection were used to examine the development and early impact of the scarring-necrosis association, and to determine if the association is related to weevil- or fungal-induced changes in plant quality.

Materials and methods

Plant and pathogen cultures

Waterhyacinth plants were obtained from an irrigation canal near Monte Alto, Hidalgo County, Texas (latitude N 26° 24.796, longitude W 97° 57.549). Plants at this site were in phenostage 2 or 3, with mostly bulbous petioles (Center et al., 1999a). Individual plants were selected and daughter plants removed. Plants were sprayed with Sevin (Tech Pac, Lexington, Kentucky) (0.03% N-methyl carbamate, 2 ml plant^{-1}) to remove natural infestations of weevils and other insects, and Daconil (Hi-Yield Chemical Co., Bonham, Texas) (0.075% chlorothalonil (tetrachloroisoph thalonitrile), 4 ml plant^{-1}) to control natural *Cercospora* infection. Untreated irrigation water supplemented with 5 ppm phosphate and 2 ppm iron (pH 6.5–7.0) was used to grow plants in a 1200 l tank equipped with a circulating pump. The water was replenished and fertilized every two weeks and changed every two months.

Cercospora piaropi was isolated from surface-sterilized leaf disks (0.5 cm) cut from plants collected at local field sites. Disks and colony transfers were cultured on solid potato dextrose agar (39 g l^{-1}) containing 5 g l^{-1} yeast extract (Difco, Detroit, Michigan) (Charudattan et al., 1985). Liquid cultures were prepared using potato dextrose broth (24 g l^{-1}) containing 5 g l^{-1} yeast. Two- to three-week-old cultures from solid and liquid media were used to generate suspensions of spores and hyphae for inoculation.

Field plot study of weevil and fungal augmentation

Eight plot groups, each consisting of four PVC plastic square plots (0.25 m^2), spaced 0.5 m apart, were placed 7 m apart in 0.75 m deep water in a reservoir located 1.5 km North of the canal where plants were collected. Seven plants were placed into each plot. Plastic screening (50% shade cloth, Kinney Bonded, Donna, Texas) stretched across the bottom of the plot supported the roots of plants, which were inserted into perforations in the mesh. An additional piece of mesh secured to

the plot was submerged with the roots to protect them from herbivores. Twenty grams of Osmocote 14:14:14 (N:P:K) fertilizer (Scotts-Sierra, Marysville, Ohio) was supplied in a mesh bag submerged in each plot. All plants were treated with insecticide and fungicide within a week after planting. All plots received foliar fertilizer spray two weeks after planting (mg nutrient plant^{-1}: Fe, 1.4; N, 0.6; K, 0.6). One plot in each group was randomly assigned to *Neochetina* spp. infestation, fungal inoculation, infestation + inoculation, and control (no augmentation) treatments. Four plants per plot were selected and the youngest unfurled leaf on each plant was tagged.

Weevil scars were defined as the light brown wounds or holes created by adult weevils chewing partially or completely through laminar leaf surfaces (Center et al., 2002). Necrotic lesions were defined as the punctate or coalescent black spots characteristic of *C. piaropi* infection (Freeman et al., 1981), and occurred both inside and outside of weevil feeding scars. Scar density was assessed by counting scars on the adaxial leaf surface and measuring the length of the lamina. A regression of laminar length to area from a trial with separate plants (Area = 6.29 (Length) – 10.4; $R^2 = 0.869$) was used to estimate scar density m^{-2} leaf area. *C. piaropi* necrotic lesion coverage on individual leaves was visually estimated as a percentage.

Waterhyacinth weevils were collected at local field sites and separated by sex and species (70% *N. bruchi* and 30% *N. eichhorniae*, consistent with local field populations (P. Moran, unpublished data)). Forty weevils (1:1 male: female) were released per plot. Insecticide was applied to protect uninfested plots. Seven days after infestation, scar densities and necrotic lesion coverage on youngest unfurled and tagged leaves were assessed on tagged plants. The disease severity (DS) for each plant was estimated with a formula modified from Charudattan et al. (1985). DS = ((number of live original leaves × gs on live original leaves) + (number of new live leaves × gs on new live leaves) + number of dead new leaves)/((Total number of live leaves) + (number of dead new leaves)), where gs = estimated percent lesion coverage for all new or original leaves. The original set of leaves was the flagged leaf and all leaves below it. All leaves above the flagged leaf were measured as the new set. The youngest expanded leaf was excised at the tip of the petiole from one untagged plant in each plot, and preserved in dry ice for protein and peroxidase analysis. *C. piaropi* (2.4×10^6 spores and hyphae ml^{-1} in 0.05% Tween-20 (Sigma-Aldrich, St. Louis, Missouri)) or mock suspension was applied at dusk to all plots. Plants were sprayed until runoff and covered for 13 h with plastic sheeting to maintain high moisture. Fungicide was applied weekly to mock-inoculated plots.

Ten days after *C. piaropi* inoculation (17 days after weevil infestation), scar density and necrosis were estimated on youngest unfurled leaves of tagged plants. Necrosis was also estimated on tagged leaves. Leaf samples for protein and peroxidase were collected as at the time of inoculation. The experiment was terminated 17 days after fungal inoculation (24 days after weevil infestation). After counting total shoot density, the four tagged plants and ten additional randomly-selected untagged plants were removed from each plot. Living and dead above-water plant parts were weighed. Scar densities on youngest leaves were determined using actual leaf areas measured with a Li-Cor 3500 leaf area meter (Li-Cor, Lincoln, Nebraska). Necrotic lesion coverage on youngest, tagged and oldest leaves and whole-plant DS were determined. The time allowed for *C. piaropi* necrotic lesion development to determine DS (17 days) was sufficient to obtain 15% or more necrotic lesion coverage in previous studies (Charudattan et al., 1985).

Weevil no-choice bioassay

Ten tank-grown waterhyacinth plants were placed in each of two plastic tanks (0.3 m × 0.8 m × 0.4 m) left outdoors and supplied with irrigation water fortified as above. Youngest unfurled leaves were tagged. *C. piaropi* or mock suspension was applied as in field plots. One-week after inoculation, 50 *Neochetina* spp. weevils (1:1 male: female, 70% *N. bruchi*) were caged with muslin netting inside the tanks and were allowed to feed for one week. Scarring and leaf areas on the new, youngest unfurled leaf, the tagged leaf (2–3 positions from the shoot apex) and the oldest live leaf were measured.

Protein content and peroxidase activity on infected plants

The youngest unfurled leaves on cultivated waterhyacinth plants were tagged and plants were inoculated with *C. piaropi*. Youngest, tagged, and oldest live leaves were excised 1, 2, and 3 weeks after inoculation from 5 to 6 plants per treatment (separate plants at each time point) and were frozen at −80 °C. These samples, and those from field plots, were homogenized (0.3 g fresh weight, FW) in 0.01 M sodium phosphate buffer (pH = 7, 10 ml g FW^{-1}, 0.75 mM EDTA and 1% polyvinylpyrrolidone). Extracts were centrifuged and 50 μl supernatant was mixed with 1.5 ml Brillant Blue G reagent (Sigma) and incubated for 5 min at 25 °C. Soluble protein content was determined colorimetrically at 595 nm relative to bovine serum albumin standard (mg g FW^{-1}). Peroxidase activity was measured using 150 μl supernatant in a total

volume of 1.5 ml containing 0.025 M phosphate buffer with 0.25% (v/v) guaiacol substrate and 0.375% (v/v) hydrogen peroxide. The change in absorbance over one minute at 470 nm was used to determine activity per g FW^{-1}.

Statistical analyses

All field plot study variables were averaged across plants to obtain one measurement per plot. DS values were Gompertz-transformed (Berger, 1981). Disease progress rates (k) were calculated by subtracting initial (0 days after *C. piaropi* inoculation) from final values (17 days after inoculation). Daily leaf production and mortality rates were determined using leaf gains and losses over 17 days. Variation in weevil scarring on youngest unfurled leaves and necrosis on all leaf ages were examined over three sampling times with repeated measures ANOVA using unstructured covariance and Type I tests in SAS PROC MIXED (SAS Institute, 1999). Univariate ANOVA and Tukey mean separation in PROC GLM examined scar density and necrosis coverage at individual times, final leaf count and fresh weight data ($n = 8$ plots per treatment), weevil feeding in the bioassay ($n = 10$ plants per treatment) and protein and peroxidase data ($n = 8$ plots or 5–6 cultivated plants per treatment). PROC CORR was used to perform Pearson correlations between scarring and necrosis measures. Transformed scar densities, protein contents and peroxidase activities ($\log(x+1)$) and necrotic lesion coverage values (arcsine-square root) were used to meet normality requirements.

Results

Weevil feeding and fungal symptom development

Prior to augmentation, youngest unfurled leaves on tagged *E. crassipes* plants were largely free of damage associated with natural *Neochetina* weevil infestation (0.02–0.07 scars cm^{-2} leaf area) and had no necrotic lesions. A substantial proportion (27%) of plants had fungal spotting on older leaves, suggesting natural *C. piaropi* infection. Weevil infestation greatly increased laminar scar density on youngest unfurled leaves across all three sampling times ($F = 32.11$, df = 3, 28, $P < 0.001$) and tagged leaves seven days after weevil augmentation ($F = 19.73$, df = 3, 28, $P < 0.001$). Scar densities on these leaves were 19–36 times greater in infested plots (0.7–1.5 scars cm^2 $area^{-1}$) than in uninfested

plots (0.01–0.10 scars cm^2 $area^{-1}$) before and 10 days after inoculation with *C. piaropi*. Densities were 5.5 times higher on plants in infested plots after one additional week, when the experiment was terminated.

Average whole-plant disease rates (k, disease severity increase day^{-1} over 17 days) were near-significantly higher in infested, inoculated plots (mean ± SE; 0.015 ± 0.009) and in plots augmented with weevils alone (0.021 ± 0.007) than in plots that received *C. piaropi* alone (0.002 ± 0.004) or no agents (−0.003 ± 0.006) ($F = 2.79$, df = 3, 28, $P = 0.06$). Necrotic lesion coverage increased over time on tagged leaves in all plots ($F = 114.5$, df = 1, 28, $P < 0.001$) (Figure 1) and coverage on tagged leaves varied over time among treatments ($F = 2.57$, df = 3, 28, $P = 0.07$). Coverage was greater on plots augmented with weevils, *C. piaropi*, or both agents than in control plots 17 days after inoculation ($F = 6.49$, df = 3, 28, $P = 0.002$) (Figure 1). Combined weevil and fungal augmentation led to the highest coverage proportions in youngest leaves ($F = 4.00$, df = 3, 28, $P = 0.02$), while plots that received weevils alone and were infected by environmental inoculum tended to have highest coverage levels in oldest leaves ($F = 2.56$, df = 3, 26, $P = 0.08$) (Figure 1). Across all treatments, scar density and necrosis coverage were correlated on youngest unfurled (Figure 2) and tagged leaves ($r = 0.48$, $n = 32$, $P = 0.006$) but not on oldest leaves ($P > 0.05$).

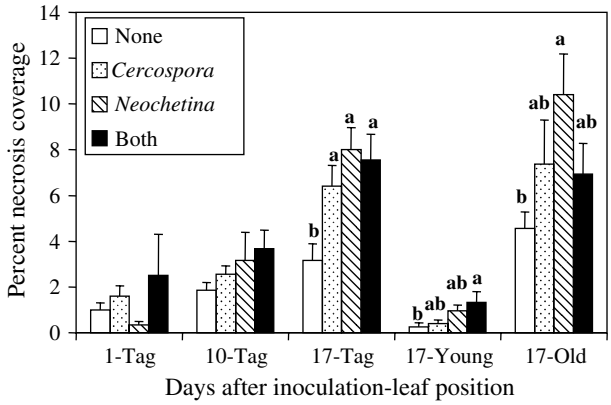

Figure 1. Necrotic lesion coverage on waterhyacinth leaves arising from *C. piaropi* infection in field plots. 'Tag–1, –10, –17', coverage on the youngest unfurled leaf measured 1, 10, and 17 days after inoculation. 'Young-17' and 'Old-17', coverage on the leaves that were the youngest and oldest live unfurled leaves 17 days after treatment. Bars represent means ± 1 SE. Means with different letters are significantly different in Tukey tests ($P < 0.05$).

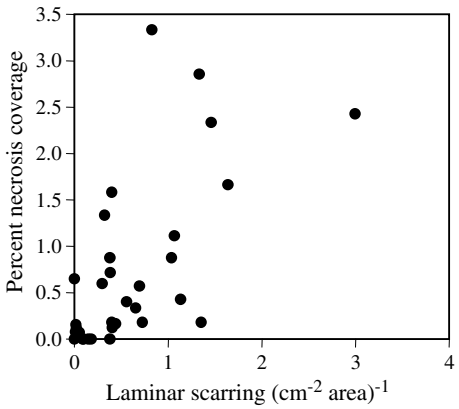

Figure 2. Correlation between laminar scarring by *Neochetina* weevils and percent leaf coverage with *C. piaropi*-induced necrosis on youngest unfurled leaves in field plots ($r = 0.68$, $n = 32$, $P < 0.001$).

Effect of augmentation on plant biomass and peroxidase

At the conclusion of the experiment (24 days after *Neochetina* infestation, 17 days after *C. piaropi* inoculation), plots augmented with both agents had 20% lower live leaf counts per plant and 38% lower plant densities than control plots (Table 1). Shoot loss from plots due to drift

Table 1. Leaf and plant production and fresh weight in waterhyacinth plants at the conclusion of the field plot study

Measure	Treament[a]			
	None	*C. piaropi* Inoculation	Neochetina infestation	*C. piaropi* and *Neochetina*
Number of leaves	5.228 ± 0.236[a]	4.960 ± 0.190[ab]	4.504 ± 0.149[ab]	4.281 ± 0.247[b]
Leaf balance[b]	−0.065 ± 0.030	−0.113 ± 0.014	−0.129 ± 0.017	−0.134 ± 0.022
Plant density[c]	86.50 ± 6.139[a]	78.00 ± 5.607[ab]	60.00 ± 8.143[ab]	54.00 ± 8.518[b]
Fresh weight[d]	29.58 ± 1.656	28.75 ± 1.554	33.52 ± 2.550	35.06 ± 5.379

[a]Plants were examined 24 days after weevil infestation (17 days after inoculation). Values are means ± 1 SE. Means with different letters are significantly different ($P < 0.05$ in Tukey tests).
[b]Leaf balance: daily leaf production – daily leaf mortality, estimated over 17 days.
[c]Density m^{-2}.
[d]Live above-water biomass (g).

was more important than shoot mortality within plots. The balance of leaf growth (daily leaf production – daily leaf mortality) and live fresh weights did not differ among plots ($P > 0.05$) (Table 1). Protein content in youngest unfurled leaves did not differ among plots. Soluble peroxidase activity was significantly higher 10 days after inoculation ($F = 9.76$, df = 3, 28, $P < 0.001$) in plots augmented with weevils and *C. piaropi* ((ΔAbs_{470} g fresh weight^{-1} min^{-1}), mean ± SE; 10.7 ± 1.94) and plots that received weevils alone (8.13 ± 1.49) compared to *C. piaropi* – only plots (5.14 ± 0.77) and control plots (3.01 ± 0.81).

Effects of fungal inoculation on plant quality

In a no-choice bioassay with plants in tanks, scarring by weevils varied significantly by leaf age and inoculation treatment ($F = 6.51$, df = 5, 54, $P < 0.001$), with higher scarring in youngest and oldest leaves (2.7 and 2.4-fold, respectively) than in tagged leaves ($F = 12.78$, df = 2, $P < 0.001$). Oldest leaves on uninoculated plants received 1.8-fold more scarring (mean ± SE; 1.56 ± 0.21 cm^2 area^{-1}) than did similar leaves on inoculated plants (0.88 ± 0.30) ($F = 5.58$, df = 1, 18, $P = 0.03$), but weevil feeding was not affected by *C. piaropi* in tagged and youngest leaves.

Soluble protein content in leaves of cultivated plants varied according to leaf age and *C. piaropi* infection three weeks after inoculation ($F = 5.44$, df = 5, 20, $P = 0.003$), but not earlier. Youngest leaves had twice as much protein as oldest leaves. Protein content was two times higher in tagged leaves on inoculated plants compared to controls (Table 2). *C. piaropi* influenced peroxidase activities three weeks after

Table 2. Protein content and peroxidase activity in waterhyacinth three weeks after inoculation with *C. piaropi*

Variable	Treatment[b]	Leaf age[a]		
		Youngest	Tagged	Oldest
Protein[c]	Control	1.847 ± 0.217	1.061 ± 0.095[b]	1.032 ± 0.067
	Inoculated	2.349 ± 0.353	2.022 ± 0.228[a]	1.230 ± 0.259
Peroxidase[d]	Control	9.460 ± 2.126	14.51 ± 1.026[b]	8.630 ± 6.970[b]
	Inoculated	7.920 ± 1.199	29.01 ± 3.353[a]	47.28 ± 8.001[a]

[a]Values are means ± 1 SE. Means in the same column with different letters are significantly different ($P < 0.05$ in Tukey tests).
[b]$n = 5$ plants for youngest and tagged leaves and 2–4 plants for oldest leaves.
[c]Protein as mg g fresh weight^{-1}.
[d]Peroxidase as change in absorbance (ΔAbs_{470}) g fresh weight^{-1} min^{-1}.

infection ($F = 13.7$, df = 5, 20, $P < 0.001$), as did leaf age at all times. Activities were elevated 2.0- and 5.5-fold, respectively, in tagged and oldest leaves on inoculated plants relative to controls (Table 2). At this time, youngest leaves on plants of both treatments had activities that were 60 and 75% lower, respectively, than tagged and oldest leaves.

Discussion

This study revealed the early stages of a positive interaction between laminar scarring and fungal necrosis development on waterhyacinth plants sequentially infested with *Neochetina* weevils and inoculated with *C. piaropi*. The brevity of the field plot study (24 days) was necessitated by homogenization of plot treatments and environmental factors. Scar density differences between weevil-augmented and control plots declined at the final sampling point, and necrotic lesions were ubiquitous on old leaves in all plots. Weevils and *C. piaropi* were thus spreading throughout the plots, despite protective insecticide and fungicide applications. The leaf balance data indicated negative leaf production in both augmented and control plots, likely caused by late-season cooling and reduction of growth (Addor, 1977; Center, 1985). Low water nutrient levels in the reservoir may have also limited growth (P. Moran, unpublished). The control plot doubling time (19 days) was within the range of published values (Gopal and Sharma, 1981). Plots were allowed to develop for 38 days (two doubling times) between planting and termination, and likely attained densities that were sufficient for biocontrol impacts on growth to be detectable.

Scar densities produced by *Neochetina eichhorniae* and *N. bruchi* were consistent with previous studies (Center et al., 1999b). Disease rates and *C. piaropi* lesion coverage were low relative to longer-term studies (Charudattan et al., 1985). Leaves in plots infested with weevils alone had DS values and symptom coverage equal to or greater than plots that received *C. piaropi* or both agents (Figure 1). The failure of fungicide application to protect non-augmented plots allowed scarring to enhance infection by environmental inoculum. Scarring and necrosis levels were correlated in the plots (Figure 2), as in field populations of waterhyacinth (Moran, 2004). A similar damage-pathogen symptom association occurs in plants stressed by waterhyacinth weevils and mites and the fungal pathogen *Acremonium zonatum* (Charudattan et al., 1978; Sanders et al., 1982; Galbraith, 1987).

Weevil augmentation alone was sufficient to induce a positive association between scarring and necrotic lesion coverage. However, Freeman

et al. (1981) concluded that releases of both weevils and the fungus are required for long-term additive effects. The magnitude of the effects of weevils and *C. piaropi* alone on plant density and leaves (Table 1) suggests that an additive biological control impact occurred in plots augmented with both agents. Fungal application may have specifically reduced the production and growth of new leaves and daughter plants, leading to effects over and above those induced by environmental inoculum on extant leaves and shoots (Charudattan et al., 1985). Decreased daughter plant production likely led to reduced stability and increased plant drift in plots that received both agents. *Neochetina* weevils (Center, 1985; Center et al., 1999a, b) and *C. piaropi* (Freeman et al., 1981; Charudattan et al., 1985) individually reduce leaf and plant survival and biomass over longer time frames.

Weevil infestation of field plots had a stronger influence than *C. piaropi* infection on soluble peroxidase activity, but induction of this antipathogenic enzyme by weevil feeding did not impede infection. The positive effects of infection on protein and peroxidase levels in tank-grown plants, while consistent with many studies in terrestrial plants (Hammerschmidt and Kuc, 1995; Hatcher, 1995) were delayed until three weeks after application, and were no greater in magnitude than leaf age effects (Table 2). Scarring by *Neochetina* spp. was elevated within two weeks of inoculation on oldest leaves. In the field, weevils show a strong preference for furled and young unfurled leaves (Center and Wright, 1991), and scarring levels did not differ on young and mid-age leaves between infected and control plants. The results clarify and strengthen previous findings that the weevil-necrotic lesion association is the result of a direct interaction, mediated possibly through enhanced fungal infection of weevil feeding sites (Charudattan et al., 1978).

This study and past work suggest that the joint presence of *Neochetina* spp. weevils and *C. piaropi* enhances biological control efficacy (Charudattan, 1984). Combined insect and pathogen damage in waterhyacinth generally increases the impact of biocontrol (Addor, 1977; Cofrancesco et al., 1985), although the effects are not always additive or synergistic (Galbraith, 1987). Manipulative (Addor, 1977; Cofrencesco et al., 1985) and observational (Center, 1987) studies of arthropods and pathogens have found that one or both of the *Neochetina* spp weevils exert the most dominant, consistent impact, and the use of these weevils alone can produce spectacular impacts (Forno and Julien, 2000; Aquilar et al., 2003). Currently, biological control does not consistently manage waterhyacinth populations in the USA, South Africa, and other temperate regions (Center et al., 2002; Coetzee et al., 2003). Novel approaches using extant released and native agents

will complement ongoing efforts to introduce new insects and pathogens. Galbraith (1987) demonstrated mechanical and digestive vectoring of *A. zonatum* fungi by waterhyacinth weevils. Although vectoring of *C. piaropi* has not been demonstrated (Charudattan et al., 1978), improved formulation technology may permit inoculation of weevils prior to their augmentative release. This approach is likely more viable than concomitant, large-scale weevil and fungal augmentation, since *C. piaropi* is not commercially available (Barreto et al., 2000). The utility of this approach should be evaluated under different seasons and stress conditions known or likely to influence the scarring-necrosis association.

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